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**BOTANY.**—*The evolutionary significance of the endosperm and its bearing on the origin of angiosperms.* HUI-LIN LI, Morris Arboretum, University of Pennsylvania.

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The process of "double fertilization," first described by Navashin (1898) and Guignard (1899) over half a century ago, is one of the most important finds in botany. It is a process peculiar to the angiosperms, involving, as is well known, the fusion of one male nucleus with the egg nucleus to form the zygote and the fusion of the second male nucleus with two polar nuclei to form the primary endosperm nucleus. The endosperm develops usually into a short-lived nutritive tissue nursing the growth of the embryo.

The study of the endosperm in the past has been largely morphological. For some time there was much discussion about its phylogenetic origin. In recent years more emphasis has been placed on its physiological and genetic significance. (Brink and Cooper, 1944, 1947; Cooper and Brink, 1944).

However, no evolutionary significance or historical explanation has so far been attached to this remarkable event in the plant kingdom. This is undoubtedly due in part to the confusion in terminology of the endosperms of gymnosperms and angiosperms. The endosperm of gymnosperms is a relatively undifferentiated tissue, essentially nutritive in function. It is, however, derived directly by the repeated cell divisions in the megagametophyte. In this respect, it is fundamentally different from the angiospermous endosperm, which is formed by the fusion of a male nucleus with one or more nuclei of the megagametophyte. In the light of modern cytology and genetics, the older view that the endosperm of angiosperms should be considered as a gametophytic tissue is un-

tenable (Brink and Cooper, 1947). To avoid confusion, it is highly desirable to restrict the term "endosperm" to angiosperms and to designate the endosperm of gymnosperms as simply megagametophyte or to use some other term for it.

There is no phenomenon in gymnosperms to suggest double fertilization and triple fusion. In addition, there are other unique features in the angiospermous endosperm. These features and their possible evolutionary and phylogenetic significance are reviewed below.

## ORIGIN

Besides one of the male nuclei, two polar nuclei take part in triple fusion. To trace the origin of the endosperm therefore means tracing the origin of the megagametophyte of angiosperms.

All proposed explanations regarding the origin of the megagametophyte of angiosperms attempt at homologizing it with that of the gymnosperms. There are three principal views (Maheshwari, 1948, 1950): that of Schürhoff (1928) which considers the megagametophyte as formed by two archegonia with prothallial tissue; another of Porsch (1907) which considers it as formed by two archegonia without prothallial tissue; and a third emphasizing the similarities between the megagametophytes of angiosperms and the Gnetales (Thompson, 1911; Fagerlind, 1941) assumes that all the nuclei possess the same value and any one of them could develop into an embryo. It is not necessary to go into detail concerning the objections to these theories (Langlet, 1927; Maheshwari,

1948) except to mention that none of these explains the origin of the fusion of the two polar nuclei and one male nucleus in the formation of the endosperm.

As Maheshwari aptly points out, "The question may well be asked as to why we should at all expect to be able to explain the angiospermous embryo sac in terms of that of the gymnosperms, when there is no evidence that the former group was derived from the latter." I am inclined to agree further with him in his statement that "It is far more likely instead that the angiosperms have long passed the stage of archegonia and probably never had them at any time in their fossil history".

#### DEVELOPMENT AND STRUCTURE

While ultimately the endosperm may become wholly or partly cellular, it originates in three main structural types, namely, nuclear, cellular, and helobial (Schnarf, 1929; Maheshwari, 1950).

The nuclear type, generally found in the supposedly primitive angiosperms, develops from the successive mitotic divisions of the primary endosperm nucleus resulting in the formation of a large multinucleate ceonocyte. This free-nucleate condition may be of short duration or may persist for several days or weeks. The number of free nuclei is usually very large, ranging from over a hundred to several thousand. Then follows cell formation and the endosperm usually becomes entirely cellular, although in some plants a central noncellular portion may persist throughout the development of the seed.

In the cellular type, nuclear divisions are accompanied by wall formation right from the beginning. Some of the cells at one or both ends may become differentiated as elongated haustorial cells which may remain uninucleate or become multinucleate.

The third type or helobial type is so called because it is of common occurrence in the Helobieae. Here the primary endosperm cell divides into two cells of unequal size: a large micropylar and a small chalazal cell. The micropylar cell becomes multinucleate and later cell formation occurs in it as in the nuclear type. The chalazal cell may remain uninucleate or may develop into a multi-

nucleate cell. The helobial type is generally considered to be intermediate between the nuclear and cellular types.

The multinucleate condition is an unique feature which does not exist elsewhere in the higher plants except in early stages of the development of the megagametophytes and also in the proembryos of the Cycadaceae and *Ginkgo*. Another peculiarity of the endosperm is the process of cell division. There are two methods of wall formation in the endosperm: by cell plates or by furrowing (Schnarf, 1929). The formation of cell plates is as in other tissues. In the following method, cleavage furrows are formed at the outer wall and advance inwardly until they meet and divide the protoplast into uninucleate cells. It is a process frequently observed in the lower plants but its occurrence in the higher plants is restricted only to pollen and endosperm formation. In pollen, however, the pollen mother cell is limited to four nuclei only. The extremely large number of nuclei formed in the angiospermous endosperm and the subsequent divisions by furrowing resulting into cellular condition has no parallel in plant groups above the thallophytes.

Aside from these distinctive characteristics, it is noteworthy that the endosperm is not only cellular but often highly differentiated structurally, while the "endosperm" of gymnosperms is more or less undifferentiated. Some of the cells usually develop into elongate haustorial cells or absorbing organs. Sometimes the cells may branch at their tips.

The endosperm persists in the seed of many species as a storage organ. While the mature endosperm generally has a more or less smooth surface, in two whole families the Annonaceae and the Myristicaceae and in many genera of other families, it is "ruminated." The significance of the latter condition is subject to further investigation.

#### TIME RELATIONS

The fusion of the two polar nuclei shortly before fertilization is also an unique feature of the angiosperms. Both the endosperm and the embryo seem to initiate more or less simultaneously at the time of double fer-

tilization. Their subsequent developments are, however, not parallel events.

The primary endosperm nucleus divides soon after fertilization and by rapid successive divisions forms an active growing endosperm within a short time. The zygote, on the other hand, usually undergoes a period of maturation before division starts. By this time there are already from several hundred to over a thousand nuclei in the endosperm. The latter may even have completed its growth entirely as in *Fouquieria* (Johansen, 1936). Only rarely does the zygote divide concurrently with the primary endosperm nucleus and the few reports that the division of the zygote precedes that of the primary endosperm nucleus have either proven erroneous or are doubtful (Brink and Cooper, 1947).

#### NUTRITION RELATIONS

The endosperm not only has a decided advantage in the time of development as compared with the embryo, but in their nutritional relationships also the endosperm occupies apparently an earlier phase than the embryo.

The endosperm is dependent on the sporophytic tissues. In the nuclear type, the nucellus furnishes the nutrition for the rapid nuclear division and expansion of the endosperm. The cellular type of the endosperm develops in an ovule that has a much reduced nucellus already absorbed by the megagametophyte. The endosperm develops absorbing haustorial cells and obtains nutritive materials from the conducting tissues in the funiculus. The large single-celled haustorium in the helobial type has a similar absorbing function.

The embryo depends in turn on the endosperm for nutrition. The endosperm is digested and absorbed during the growth of the embryo. After the cotyledons are formed, they digest their way through the endosperm and absorb it until it nearly disappears. Only remnants of the endosperm are usually left in the mature seed, the cotyledons becoming storage organs. In some species the endosperm persists as storage tissues while the cotyledons store little or no nutritive materials.

Recent studies on the cultivation of excised embryos *in vitro* have shown that, during the early period of development of the embryo, there is an unique physiological relationship between embryo and mother plant through the endosperm. The mother plant is only indirectly host to the offspring through the medium of the endospermous tissue. Cultivation of very young embryos is extremely difficult and few investigators have succeeded in growing seedlings from them. The young embryo is, as stated by Brink and Cooper (1947), initially incapable of performing certain essential growth functions, and these functions are mediated for the embryo by the endosperm.

#### CHROMOSOME NUMBER

The endosperm of gymnosperms, since it is derived by continuous cell division of the haploid megaspore, is haploid in its chromosome number. In the angiosperms, the endosperm is triploid, having a diploid set from the two polar nuclei and a haploid set from the male nucleus. The triploid endosperm is an unique structure characteristic of the angiosperms. Not only nothing comparable is found in the gymnosperms, but also in the ferns, the "fern-allies", and the bryophytes as well. The triploid nature of the endosperm indicates that it is neither sporophyte tissue with  $2n$  chromosomes, nor gametophyte tissue having  $n$  chromosomes.

On chromosome number alone, there is no basis to homologize the so called endosperm of gymnosperms with the endosperm of angiosperms. The two tissues are only similar in their function in nursing the developing embryo. This distinct product of syngamy must have a more fundamental and profound significance in the history of angiosperms than merely the provision of a nutritive tissue to the developing embryo. This may well be a collateral and incidental function, especially as maternal sporophytic tissues are readily available to perform such a function.

A phylogenetic interpretation of the alternation of generations in plants has been offered by Allen (1937). He considers that a regular alternation of generations in green and brown algae and possibly also in the ancestors of pteridophytes and of seed

plants, seems to have been established by a diploid duplication of the haploid generation. While in long-cycled red algae, alternation came about through duplication as well as transfer, the latter referring to the transference of the post-zygotic phase from the haplont to the diplont. Among other things, he compares the extremely reduced haploid generation in the angiosperms with that of *Fucus*. The reduction series as represented in seed plants is compared with parallel series in algae. Allen's interpretation, given in the light of some obvious genetic considerations, is a valuable contribution to a much neglected subject. However, he does not mention anything about the endosperm. Taking the latter into consideration, the life cycle of the angiosperms, following Allen's argument, probably approaches more closely the red algae in general than other plant groups.

#### GENETIC RELATIONS

Genetically, the endosperm differs from the embryo in that it receives a double complement of inheritance from the megagametophyte. As a result of triple fusion, the angiospermic seed is unique in being genetically a most diverse structure. The normal chromosome ratio between the maternal sporophytic tissues, the endosperm and the embryo is 2:3:2. The successful formation of the seed thus depends on the maintenance of the genetical balance of the three components. Seed collapse in interspecific hybrids is generally believed as due primarily to embryo disfunction. However, recent studies, as summarized by Brink and Cooper (1947), have shown that impairment in development or death of the embryo is frequently due to the failure of the endosperm formation. Young embryos in abortive seeds with underdeveloped endosperm can sometimes be excised and cultured artificially into seedlings (Sawyer, 1925; Werekmeister, 1937; Lenz, 1954).

That the endosperm behaves genetically differently from the embryo can be further illustrated by the phenomenon known as xenia, namely, the effect of foreign pollen on visible characters of the endosperm, of which there are several examples in maize (Emer-

son *et al.*, 1935). This is due to the fact that the two fertilizations occurring within a single megagametophyte occasionally involve participation of male nuclei from different pollen grains, a phenomenon called "heterofertilization" by Sprague (1932).

Thus double fertilization actually involves two distinct processes which may occur independent of each other. There are occasional cases of plump grains found among cereals without embryos. The immediate cause of these embryoless grains is assumed to be single fertilization whereby only the endosperm develops (Brink and Cooper, 1947). All these observations show that both the genotype and phenotype of the endosperm are independent of the constitution of the embryo with which it is associated.

#### ENDOSPERM AND THE ORIGIN OF ANGIOSPERMS

As the embryo is directly in the line of descent, it is necessarily the cardinal component of the seed. Nevertheless, it is now clearly demonstrated that the endosperm is primarily responsible for the maintenance of the continuity of the life cycle of the plant at the early seed stage. During this critical period, the embryo is dependent entirely on the endosperm for performing certain growth functions.

Brink and Cooper (1947) are of the opinion that "the double complement of inheritance perceived by the endosperm from the maternal parent is an adaptation which facilitates functioning of the endosperm in its intercalary position between mother plant and embryo. The secondary fertilization is thus interpreted as a method to confer on the tissue the physiological advantage in reproduction associated with the extreme reduction of the female gametophyte".

Compared with the gymnosperms which have a massive megagametophyte readily available to nourish the embryo at the time of fertilization, the simple megagametophyte of angiosperms is in a decidedly disadvantageous position. As a device to compensate this deficiency, the endosperm performs the function very well. But the problem still left unexplained is the *de novo* appearance of this unique and complicated phenomenon of double fertilization and triple fusion.

As mentioned before, I am inclined to agree with Maheshwari (1948) in that the origin and development of the megagametophyte of angiosperms cannot be explained in terms of the gymnosperms. Similarly, the origin of the angiospermous endosperm, as attested by its many distinct characteristics discussed before, also cannot be sought among the gymnosperms. An historical explanation of the origin of the endosperm has never been offered.

From an evolutionary point of view, the origin of the angiospermous endosperm cannot be sought among the bryophytes, the ferns and the so called fern-allies, as well as the gymnosperms, for any comparable structure is not found among these groups of plants, at least among all the living forms.

On the other hand, among the algae and fungi, the life cycles of many plants involve three or more distinct phases, such as among the red algae and in many fungi. Sometimes two of the phases may develop almost simultaneously. The life cycle of angiosperms is thus reminiscent of the situation common in the thallophytes. The fusion of the two polar nuclei is also a unique process but more or less similar conditions are also found in many of the algae and fungi, such as in the formation of teliospores in rusts (*Uredinales*) and chlamydospores in smuts (*Ustilaginales*), where a binucleate condition eventually becomes uninucleate. An exceptionally similar condition to triple fusion is found among the *Florideae*, where a diploid connecting cell is fused with a haploid axillary cell, though in this case the nuclei concerned are probably not actually united. (Fritsch, 1945). Moreover, the most unique morphological feature of the endosperm, the large multinucleate body with the furrowing method of cell formation, also cannot be traced to the immediate lower groups of plants, but it occurs also only in some algae and fungi. It is especially common in the development of the sporangia such as in the brown algae like in the *Ectocarpales* and the *Laminariales* and in fungi like in the *Saprolegniales* and the *Mucorales*.

In my opinion, the origin of the endosperm has important bearings on the larger problem of the origin of the angiosperms. It seems futile to seek the origin of angiosperms in some groups of plants that offer no clue to the origin of the endosperm. The evolutionary significance of the endosperm deserves critical evaluation. The many distinct features mentioned above indicate relationships traceable from the angiosperms more or less directly toward the thallophytes but not through the ferns or gymnosperms. The angiosperms apparently represent a line of development parallel to that of the ferns and gymnosperms from a general algal an-

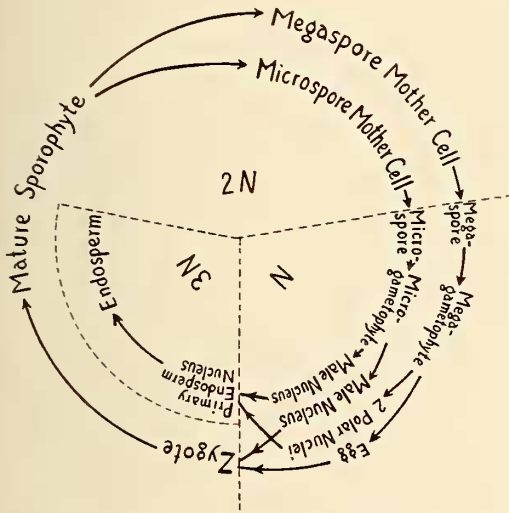


FIG. 1.—Life cycle of an angiosperm.

The triple fusion and the consequent triploid condition of the endosperm, in my opinion, clearly indicate that it is an equivalent to the gametophytic and sporophytic generations. Morphologically, physiologically, cytologically, and genetically as discussed before, the endosperm is distinct from both the sporophyte and the gametophyte. In other words, the life cycle of angiosperms should be interpreted anew as constituting three instead of only two distinct phases, the third one represented by the endosperm (Fig. 1).

While the endosperm and the embryo begin more or less simultaneously at the time of double fertilization, the precocious development of the endosperm, the strict dependence of the embryo on the latter in its early life, as well as the relative position of the two seem to suggest that the endosperm represents a slightly prior phase in their sequence of development.

cestry, but not a derived group originated from any of the gymnosperms.

#### CONCLUSIONS

This paper is presented primarily to propose a different approach for research toward the problem of the origin of angiosperms. It calls attention to a neglected field of study that seems to offer great possibilities. As yet our knowledge concerning the endosperm is very limited. With new developments in technique such as tissue culture and advancement in some fields of knowledge such as morphogenesis, physiological genetics, and others, the study of endosperm can be of great value in interpreting the evolution and phylogeny of the plant kingdom. Lacking fossil evidence, discussions at present on the origin of angiosperms are still largely a matter of conjecture. To quote the words of the late Sir Albert Seward (1921), commenting on the origin of angiosperms, "While admitting our inability at present to do more than suggest possibilities, we may encourage research by speculation".

#### SUMMARY

To summarize, the following point of view is suggested as a working hypothesis toward the problem of the origin of angiosperms. The endosperm represents, in addition to the gametophytic and the sporophytic generations, a third phase in the life history of the angiosperms, possibly the remnants or a much more reduced form of a formerly more elaborate structure. Since a similar structure is not found in the life histories of the gymnosperms and other lower groups of plants, the origin of the angiosperms probably lies in some extinct groups of plants derived directly from the thallophytes.

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